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Release and co-release of model hydrophobic and hydrophilic actives from 3D printed kappa-carrageenan emulsion gels

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Abstract

This study formulated and compared 3D printed (3DP) and cast kappa-carrageenan (κ C) emulsion gels for the co-release of model lipophilic (cinnamaldehyde) and hydrophilic (erioglaucine disodium salt (EDS)) molecules. Tween 20 (T20) or whey protein isolate (WPI) were used as the emulsifier. Both 3DP and cast emulsion gels maintained their oil droplet size over 8 weeks owing to the set gel matrix. Penetration texture analysis revealed 3DP and cast 5% oil emulsion gels, required more force to break compared to 40% oil gels (3 N against 0.4-0.5 N). This was because the oil droplets, disrupted the gel matrix; thereby weakening it. 3DP gels required less force to break than cast gels, owing to failure between the printed layers. Release tests in various media showed no significant difference in the final % cinnamaldehyde released between 3DP gels and cast gels. Release tests in carried out 0.1M hydrochloric acid saw an increase in cinnamaldehyde release compared to other media, owing to cinnamaldehyde's increased solubility in acidic media. Addition of EDS into the gel matrix facilitated co-release studies, with EDS release having no effect on the cinnamaldehyde release, indicating EDS release was driven by liberation from the gel network and cinnamaldehyde release by its expulsion from the oil droplets. Simple modelling showed that diffusion rather than polymeric relaxation was more dominant for active release in 3DP gels compared to cast gels. This work shows that 3DP can be used to produce customisable κ C-emulsion gels, with multiple actives; suitable for use as modified release vehicles.

1. Introduction

3D-printing (3DP), otherwise known as additive manufacturing, is a production technique that uses digital files rendered slice by slice to create products in a layer-by-layer manner. Initially 3DP was primarily concerned with construction materials such as plastic polymers (Rahim, Abdullah, & Md Akil, 2019), ceramics (Z. Chen, et al., 2019) and metals (Buchanan & Gardner, 2019). However, 3DP research has expanded to encompass various other research avenues including pharmaceuticals (Goyanes, et al., 2017), and edible ingredients such as dough (Fan Yang, Zhang, Prakash, & Liu, 2018), dairy gels (Daffner, Ong, Hanssen, Gras, & Mills, 2021; Daffner, Vadodaria, et al., 2021), chocolate (Lanaro, Desselle, & Woodruff, 2019) and many more, via a variety of methods (Gholamipour-Shirazi, Kamlow, T Norton, & Mills, 2020). 3DP of food is an area of much research interest owing to its ability to create highly customisable products at the point of consumption or sale, and make alterations without the need for moulding or tooling (Sun, et al., 2015). As things stand, there still exist process disadvantages compared to current mass production techniques, including high cost per individual item, low-throughput and a restricted repertoire of printable edible materials; which still require further research (Pallottino, et al., 2016).

43 One of the major issues within the area of food 3DP is undoubtedly the multifaceted nature of
44 food systems, comprising multiple phases in varying ratios, micro- and nanostructure factors
45 that affect structural and sensory traits and sensitivity to processing, ion and temperature
46 changes (Ubbink, Burbidge, & Mezzenga, 2008). Hydrocolloid gels (hereafter referred to as
47 hydrogels) have become an area of much interest within the field of 3DP. Hydrogel
48 formulations commonly consist of 0.1-10% w/w hydrocolloid, small quantities of salts and
49 water. Therefore, there is much scope for flexible formulations using hydrogels as a starting
50 base. This can involve the addition of sugars, salts and other hydrophilic molecules (Ricci,
51 Derossi, & Severini, 2019). Past 3DP studies into hydrogels have focused on agar (Serizawa,
52 et al., 2014), starch (Huan Chen, Xie, Chen, & Zheng, 2019), alginate (Jin, Compaan,
53 Bhattacharjee, & Huang, 2016) and mixtures of materials (Fenton, Gholamipour-Shirazi,
54 Daffner, Mills, & Pelan, 2021; Z. Liu, Bhandari, Prakash, Mantihal, & Zhang, 2019; Vadodaria,
55 He, Mills, & Wildman, 2020)

56 One biopolymer of specific interest in hydrogel 3DP is kappa-carrageenan (κ C). κ C is an
57 anionic, sulphated polysaccharide extracted from an edible seaweed named Rhodophyta.
58 When dispersed in water, alongside complementary gelling cations, κ C forms firm, thermally
59 reversible gels. The most effective gelling cation for κ C is potassium (Hermansson, Eriksson,
60 & Jordansson, 1991). When the hot dispersion is lowered below its gelation temperature (T_{gel}),
61 random κ C coils order themselves into double helices, and these aggregate to form a
62 polymeric network (Norton, Morris, & Rees, 1984). κ C has been used as a hydrogel feed
63 source for cold-extrusion 3DP (Gholamipour-Shirazi, Norton, & Mills, 2019), hot extrusion 3DP
64 by itself (Diañez, et al., 2019), and as part of a mixed-system (Gholamipour-Shirazi, Norton,
65 & Mills, 2021).

66 The need to produce more complex feeds for 3DP has turned attention to emulsion gels,
67 involving the production of an oil-in-water (o/w) emulsion and then dispersing gelling agents
68 within the water phase. These gels more closely resemble real food systems compared to
69 simple water based hydrogels. This facilitates the production of more complex and
70 customisable products and enables the straightforward incorporation of lipophilic molecules.
71 Since 3DP has the potential to customise food assemblies through precise ingredient
72 placement and distribution, emulsion gels are ideal candidates for further study. Their uses
73 include modification of food texture (Matsumura, Kang, Sakamoto, Motoki, & Mori, 1993) and
74 delivery of lipophilic molecules both alone (F. Liu & Tang, 2016; Thakur, et al., 2012; Zhang,
75 et al., 2022) and alongside hydrophiles (Singla, Saini, Joshi, & Rana, 2012). There has been
76 some study into 3DP of emulsion gels (Hu, et al., 2019; Johannesson, Khan, Hubert, Teleki,
77 & Bergström, 2021; Wang, et al., 2021), but as far as the authors are aware none involving
78 κ C-emulsion gels apart from a previous study (Kamlow, Spyropoulos, & Mills, 2021). There is
79 currently an increasing interest in the formulation of multi-dose medicines and nutritionally
80 fortified foods (David Julian McClements, 2018; Nagula & Wairkar, 2019). Therefore emulsion
81 gels are an obvious area for research in the sphere of functional foods, owing to their biphasic
82 nature, and ability to manipulate energy levels through oil content. However, despite this, none
83 of the previous studies assessed the release of a model lipophile (cinnamaldehyde) with and
84 without a model hydrophile (Erioglaucine disodium salt) compared to the cast equivalent. Even
85 though previous studies have shown that by moving from cast to 3DP bulk structures, you can
86 affect a change in release rates, while providing a far greater degree in flexibility with respect
87 to dosage size, shape and appearance (Kamlow, Vadodaria, Gholamipour-Shirazi,
88 Spyropoulos, & Mills, 2021).

89 Cinnamaldehyde is an essential oil extracted from the bark of cinnamon trees. It has
90 antibacterial and antifungal properties (Gill & Holley, 2004; Siddiqua, Anusha, Ashwini, & Negi,
91 2015) as well as antioxidant activity (Gowder & Devaraj, 2006). Cinnamaldehyde has a log P

92 of 1.82, meaning that it has very limited water solubility, but retains enough to be able to track
93 the release over time from a lipophilic environment to a hydrophilic one (Ben Arfa, Preziosi-
94 Belloy, Chalier, & Gontard, 2007). Cinnamaldehyde has been utilised in release studies
95 before, and is an optimal model lipophile owing to its ability to partition from an oil phase into
96 a water phase, pleasant aroma and safety profile, being generally recognised as safe by the
97 food and drug administration (Govindaraj, Subramanian, & Raghavachari, 2021). Erioglaucine
98 disodium salt (EDS) is a water-soluble dye that once dissolved gives a blue colour and is often
99 used in release studies as a model hydrophilic molecule (Andrews, et al., 2009; Jeong, et al.,
100 2021; Lu, Tarn, Pamme, & Georgiou, 2018).

101 The present study aims to evaluate the release of model lipophilic with and without hydrophilic
102 small molecular weight species from hot extrusion 3DP κC-emulsion gels, and assess their
103 performance compared to the equivalent cast gels, in order to assess the performance of both
104 as possible solid dosage forms or implants. This involved formulating 3% w/w κC-emulsion
105 gels containing either 5% or 40% w/w sunflower oil (SFO) and stabilised with Tween 20 (T20)
106 or whey protein isolate (WPI). After production of simple o/w emulsions with and without
107 cinnamaldehyde, with a monomodal or bimodal distribution, droplet size was tested through
108 dynamic light scattering and emulsion stability was scrutinised via zeta-potential
109 measurements. κC-emulsion solutions were created by dispersing κC powder into the simple
110 emulsions while heating. Following this, κC-emulsion gels were created either by 3DP or
111 casting in moulds. 3DP and cast κC-emulsion gels had their stability tested through droplet
112 size measurements utilising time domain nuclear magnetic resonance (NMR) spectroscopy
113 and through syneresis measurements. The gels' mechanical properties were also scrutinised
114 through texture profile analysis, namely penetration tests, comparing 3DP and cast gels.
115 Finally, the printed and cast gels underwent release tests to assess their performance as
116 release vehicles in various release media, with cinnamaldehyde release examined. After this
117 EDS was loaded into the gels as a model molecule to test hydrophilic release and co-release
118 studies were carried out.

119

120 **2. Materials and methods**

121 **2.1. Materials**

122 κC, T20, cinnamaldehyde, potassium chloride (KCl), and EDS were purchased from Sigma-
123 Aldrich (UK). High performance liquid chromatography grade pentane and 32% w/v HCl were
124 purchased from Honeywell, (UK). Phosphate Buffer Solution (PBS) tablets were obtained from
125 Oxoid (UK). WPI was obtained from Sachsenmilch Milk & Whey Ingredients (Sachsenmilch
126 Leppersdorf GmbH, Wachau, Germany). According to the manufacturer it contained 93.74%
127 w/w protein in dry matter, 0.23% w/w fat, 0.61% w/w lactose and 3.16% w/w ash. SFO was
128 purchased from the supermarket Spar (UK). Milli-Q water was used (Elix® 5 distillation
129 apparatus, Millipore®, USA) for sample preparation. All materials were used as received with
130 no further modification or purification.

131

132 **2.2. Emulsion preparation**

133 Simple emulsions containing no κC were produced for particle size analysis before gelation
134 and zeta-potential measurements. Production of the emulsions followed the same procedure
135 as that previously described by (Kamlow, Spyropoulos, et al., 2021). Emulsions stabilised with
136 T20 had 1% w/w T20 added to the required amount of water and SFO. Emulsions stabilised
137 with WPI had a 2% w/w stock solution of WPI diluted to 1% w/w WPI by adding deionised
138 water and SFO. These mixtures were then premixed on a Silverson L5M for 3 minutes at

139 6000 rpm with a fine emulsor screen. The formed pre-emulsions were then passed through a
140 high-pressure homogeniser at 25 bar to produce smaller scale emulsions with a droplet size
141 of approximately 1 μm and 1000 bar to produce larger-scale emulsions with a droplet size of
142 approximately 8-18 μm . Emulsions containing cinnamaldehyde had 0.7% w/w of the SFO
143 fraction replaced with cinnamaldehyde and these were stirred together using a magnetic stirrer
144 for ten minutes to ensure complete mixing. To create bimodal droplet size distribution
145 emulsions, larger and smaller scale emulsions were created and then mixed 50/50 by stirring
146 with a magnetic stirrer for five minutes.

147

148 **2.3. κC -Emulsion solution preparation**

149 T20 and WPI stabilised O/W emulsions (as described in section 2.2) with and without
150 cinnamaldehyde were used for the preparation of κC -emulsion solutions as described by
151 (Kamlow, Spyropoulos, et al., 2021). Emulsions were heated for 30 minutes on a hot-plate
152 stirrer set to 80 $^{\circ}\text{C}$. Then κC was added and left to stir for two hours to ensure that all κC had
153 dispersed. 3% w/w κC in the water phase was chosen as this has been reported to give optimal
154 printing outcomes (Kamlow, Spyropoulos, et al., 2021; Kamlow, Vadodaria, et al., 2021).
155 Finally, O/W emulsion gels were formed by cooling the systems, either via 3D printing as
156 described in section 2.8 or casting in moulds as described in section 2.9.

157

158 **2.4. κC -EDS-emulsion solution preparation**

159 For the co-release tests, κC -emulsion solutions containing EDS were prepared to test the
160 release of EDS by itself. Emulsions were produced as in section 2.3, but with 7.04g of water
161 removed. Then after κC -emulsion production, 7.04g of EDS 2% w/w solution was added to
162 the hot κC -emulsion solution and stirred for a further 30 minutes. This solution could then be
163 gelled via 3D printing as in section 2.8 or casting in moulds as in section 2.9.

164

165 **2.5. κC -EDS-emulsion with cinnamaldehyde solution preparation**

166 For the co-release tests, κC -EDS-emulsion solutions containing cinnamaldehyde were
167 produced by following the method in 2.4 but utilising an emulsion that had 0.7% w/w of the
168 SFO fraction replaced with cinnamaldehyde.

169 **2.6. Simple emulsion droplet size analysis**

170 The emulsion droplet size was obtained using a Malvern Mastersizer MS 2000, (Malvern
171 Panalytical, UK), utilising a Hydro SM manual small volume sample dispersion unit. This gave
172 data for Surface weighted mean ($D_{3,2}$) and volume weighted mean ($D_{4,3}$) droplet size values
173 were obtained immediately after preparation. The values for refractive index were input into
174 the software and were 1.33 for water and 1.467 for the sunflower oil. For the SFO mixed with
175 cinnamaldehyde, a refractometer (J357 Automatic refractometer, Rudolph research) was used
176 to calculate the refractive index of the mixture. Cinnamaldehyde was found to have a refractive
177 index of 1.62, and the mixture of cinnamaldehyde and sunflower oil was found to be 1.468.
178 Samples were dispersed in distilled water at 1300 rpm to give an obscuration value of 4.2-
179 4.6%. Samples were prepared and tested in triplicate and droplet size values were the average
180 of at least three measurements.

181

182 **2.7. Zeta-potential measurement**

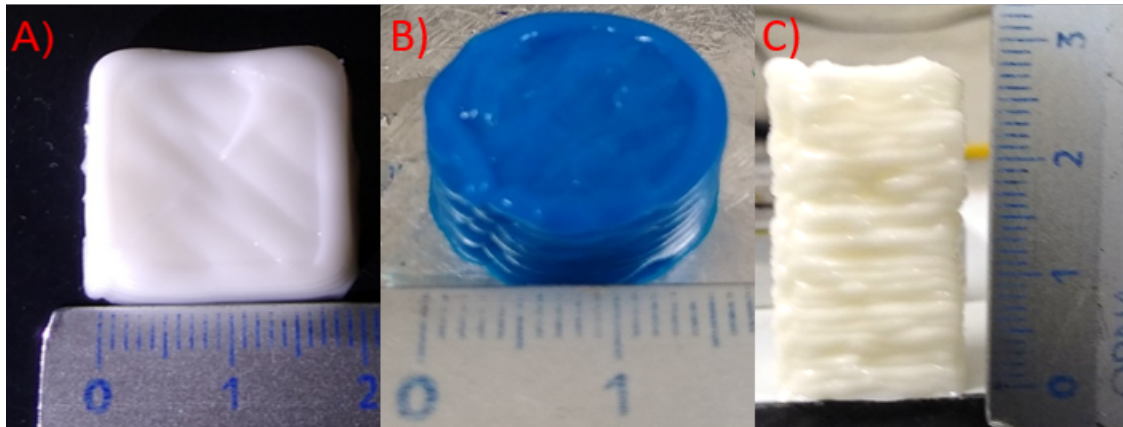
183 The zeta potential (ζ -potential) was determined using a Zetasizer (Malvern Panalytical, UK) in
184 order to assess the stability of the emulsions created and to assess if replacing part of the
185 SFO fraction with cinnamaldehyde affected stability. Samples were diluted 100 times with
186 deionised water (Wu, et al., 2016). This was to reduce the absorbance of laser light and
187 multiple scattering. All ζ -potential measurements were carried out at room temperature.

188 2.8. 3D printing

189 The 3D printer was supplied by the Institute of Food Science and Biotechnology at the
190 University of Hohenheim (Germany). It is a Fabbster 3D printer that was modified in order to
191 handle a liquid feed. Full detail of the printer's setup and function can be found in a previous
192 study (Kamlow, Spyropoulos, et al., 2021). After production of κ C-emulsion solutions as in
193 sections 2.3-2.5, these were fed into the printer, with the temperature being maintained
194 indirectly via double walled pipes containing heated water in counterflow. The syringe was
195 also wrapped in a heated jacket to prevent pre-gelation. The computer would then slice the
196 desired 3D printed shape into layers and send this information to the printer which would then
197 move in the X, Y and Z axes to produce the 3D shape. The material flowed via a syringe driver
198 with a controllable flow rate, which was important as 5% w/w SFO and 40% w/w SFO emulsion
199 solutions had different viscosities, necessitating two different flow rates (Pal & Rhodes, 1989).

200 The printing parameters for these emulsion gels were determined in a previous study
201 (Kamlow, Spyropoulos, & Mills, 2021). The replacement of 0.7% of the SFO with
202 cinnamaldehyde, as well as the addition of 0.1408% w/w EDS in the water phase had no effect
203 on the printability of the gels which were printed at the optimal parameters previously defined.
204 These were a printer bed temperature of 35 °C, print speed of 30 mm/s, fill space of 1.25 mm,
205 a water bath temperature on the feed pipes of 72 °C, a water bath temperature on the nozzle
206 of 72 °C, layer height of 1.2 mm, nozzle size of 20G/0.8mm and nozzle height of 0.5 mm. The
207 syringe driver rate was 0.7 mL/min for 5% SFO κ C-emulsion gels and 0.84 mL/min for 40%
208 SFO κ C-emulsion gels. This was used to produce 20 x 20 x 9 mm cuboids for texture profile
209 analysis and syneresis testing, 15 x 15 x 30 mm cuboids for droplet size testing and 15mm
210 height and 12 mm diameter cylinders for release tests. These are shown in Fig 1.

211



212

213 *Figure 1: 3DP (A) 20 x 20 x 9.6 mm cuboid, (B) Cylinder containing EDS and (C) 15 x 15 x 30 mm cuboid*

214

215 2.9. Production of moulds for casting

216 Cylinder and cuboid shaped moulds were produced using a form 2 stereolithography 3D
217 printer (Formlabs, USA). The moulds were designed by computer aided design and uploaded
218 to the software, digitally sliced into layers, then transmitted to the printer digitally to print. The
219 cube mould facilitated the production of emulsion gel cuboids with dimensions of 20 x 20 x 9.6
220 mm by casting. These were then used for comparative penetration testing as a control, cast
221 sample to compare to the 3D printed cuboids produced in 2.8. The cylinder mould was used
222 to produce cast, control samples for release tests of cinnamaldehyde, EDS or both at the same
223 time, to compare to the 3D printed cylinders produced in 2.8.

2.10. Syneresis testing

KC emulsion gel cuboids containing 5% or 40% SFO with dimensions of 20 x 20 x 9.6 mm were produced by 3D printing or casting and stored in an airtight container at 5 °C. Syneresis measurements were carried out by measuring the amount of water release by the emulsion gels after several time intervals as described in a previous study (Ako, 2015). The syneresis ratio (R_s) was determined using equation 1.

$$R_s = \frac{w_e}{w_g} \times 100 \quad \text{Eq [1]}$$

Where w_e is the weight of water released by the gel, and w_g is the weight of the initial gel. Since 40% SFO emulsion gels contain considerably less water per 100g compared to 5% SFO emulsion gels, the values were normalised to a constant water concentration.

2.11. Time-Domain nuclear magnetic resonance spectroscopy

Droplet size measurements of the emulsion gels were performed using an NMR device (Bruker Minispec NMR, Bruker Optics, UK), equipped with a gradient unit. Measurements were performed on three different samples, in triplicate. Droplet size calculations were performed using the Minispec software, which fits the data to a log-normal curve. Measurements were taken to assess the stability of the emulsion gels over 8 weeks, with samples tested at 0, 1, 2, 3, 4 and 8 weeks. Cast gels were tested by adding hot κC-emulsion gel solution to the NMR tubes. 3DP gels were tested by printing a cuboid measuring 15 x 15 x 30 mm and then using a cork borer, the same size as the internal diameter (Ø 10mm) of the NMR tubes to cut out a cylinder and place that into the NMR tube. $D_{3,2}$ values from section 2.6 were converted to the volume-weighted geometric mean diameter ($D_{3,3}$) values to be used as a comparison to see if oil droplet sizes changed during gelation, using equation 2 insert reference on endnote - <https://doi.org/10.1006/jcis.2001.7603>.

$$D_{3,3} = \frac{D_{3,2}}{e^{-\sigma^2+2}} \quad \text{Eq [2]}$$

where $D_{3,3}$ is the geometric weighted mean diameter, $D_{3,2}$ is the surface weighted mean diameter and σ is the standard deviation of the logarithm of the droplet diameter.

2.12. Texture Profile Analysis

Texture profile analysis (TPA) was carried out using a TA XT plus Texture Analyser. Printed and cast cuboids of dimensions 20 × 20 × 9.6 mm were tested; the cast cuboids were given 30 minutes to set, mimicking the time taken to print their respective counterparts. Penetration testing was carried out using a P/6 cylindrical aluminium probe set to a constant speed of 0.5 mm/s, over a distance of 6 mm, alongside a 30 kg load cell and 3 g of trigger force. Through penetration testing, data for the force at breaking, firmness and gel strength were acquired for printed and cast cuboids. Force at breaking, in g, is defined as the first significant discontinuity produced in the curve during penetration (Fizman, Lluch, & Salvador, 1999). Firmness, in g/mm, is the initial slope of the penetration curve within the first 2 seconds (Fizman & Salvador, 1999). Gel strength, in g x mm, is the multiplication of the penetration force by the distance of the penetration where failure occurs (H. Liu, Nie, & Chen, 2014). All tests were carried out in triplicate.

2.13 Release studies

Release studies were carried out using ultraviolet (UV)-visible spectrophotometry to assess the release of cinnamaldehyde from the printed and cast κC-emulsion gels. All studied gel systems were of a consistent weight; 1700 mg ± 5%. Oil droplets were tested at both the micron and sub-micron scale. Three cast and three printed κC-emulsion gels were each placed inside semipermeable cellulose dialysis membrane (approx. 80 mm x 40 mm), which had been

271 soaked for 24 hours in deionised water. The molecular weight cut-off for the membrane was
272 stated to be 14,000 Daltons. This was far below the molecular weight of the κC, ensuring none
273 would interfere with the absorbance readings (Phillips & Williams, 2009). The gel-containing
274 membranes were placed within 150 mL of various media (deionised water, PBS, 0.1 M HCl
275 and 1 M KCl). 100% release of the cinnamaldehyde within the gels would equate to
276 approximately 4 mg/L. Since this is significantly lower than the maximum solubility within
277 water, this equates to sink conditions. The release tests were carried out within an Incu-Shake
278 MIDI shaker incubator (Sciquip, UK) at 150 rpm. Release tests were carried out at 37 °C for
279 *in vitro* testing. Measurements were taken at various time points up to and including 360
280 minutes. Determination of the cinnamaldehyde release was carried out using a Jenway
281 Genova Bio life science spectrophotometer (Cole-Parmer, UK), set to 290 nm, the maximum
282 absorbance for cinnamaldehyde in the various release media. 0.9 mL of dissolution medium
283 was taken with an Eppendorf pipette and tested at the times stated above. This was placed
284 into the UV-Vis spectrophotometer which had been blanked using fresh release media. The
285 release profile was calculated from a calibration curve determined by the UV-Vis
286 spectrophotometer, which had an R² value of 0.999. The contents of the cuvette were then
287 discarded and 0.9 mL of fresh medium added into each beaker. This was corrected when
288 determining the cinnamaldehyde release percentage following the procedure of (B. Singh,
289 Kaur, & Singh, 1997). All tests were carried out in triplicate.

290

291 **2.14 Co-release studies**

292 Co-release studies were carried out in water using a modified version of the protocol in 2.13.
293 KC-EDS-emulsion gels with cinnamaldehyde were cast and printed, placed within
294 semipermeable cellulose dialysis membrane, then placed into beakers containing 150 mL
295 water. 100% release of the EDS would equate to approximately 8 mg/L, which meant that it
296 was being released into sink conditions. 1.8 mL aliquots were taken at the various release
297 points. 0.9 mL was taken and tested for EDS release at 629 nm on the UV-vis
298 spectrophotometer, which had been blanked against deionised water. The release profile was
299 calculated from a calibration curve for the EDS which had an R² value of 0.999. Then a solvent
300 extraction protocol was carried out on the remaining 0.9 mL. 0.9 mL of pentane was added to
301 the remaining 0.9 mL of the aliquot and they were shaken together. The cinnamaldehyde was
302 far more soluble in the pentane than the water, but the EDS is insoluble in the pentane
303 (Balaguer, et al., 2013). This prevented any interference from the EDS when measuring the
304 absorbance of the cinnamaldehyde in the pentane. This was tested within the UV-vis
305 spectrophotometer at 280 nm, as being within pentane caused a shift in the maximum
306 absorbance peak compared to being dissolved within water. Blank pentane that had been
307 shaken through deionised water was used to blank the instrument. A separate calibration
308 curve was created for cinnamaldehyde in pentane which had an R² value of 0.990.

309

310 **2.15 Modelling of release data**

311 Cinnamaldehyde and EDS release data (up to 60%) were fitted to the following model (Ritger
312 & Peppas, 1987)

$$313 \frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \quad \text{Eq [3]}$$

314 where M_t / M_∞ is the fraction of active released at time t . The first term ($k_1 t^m$) relates to Fickian
315 effects while the second term ($k_2 t^{2m}$) to relaxational contributions to the release. k_1 is the kinetic
316 constant regarding release from the matrix by Fickian diffusion and k_2 is the kinetic constant
317 for case-II relaxation. Lastly the coefficient m is the purely Fickian diffusion exponent which is
318 dependent on the shape of the device; the value of the exponent concerning relaxation
319 transport is in theory twice the Fickian exponent ($2m$). This modelling yielded values between
320 0.5 and 1. The closer to 0.5, the greater the contribution of Fickian diffusion to the release of
321 the molecules. The closer to 1.0, the greater the contribution of polymeric relaxation to
322 molecular release.

323 2.16 Statistics

324 The average droplet size, ζ -potential, R_s , force at break, gel strength, firmness and cumulative
325 release % were compared using the two-sample T-test in the Analysis ToolPack for Microsoft
326 Excel. Confidence levels were set at 95%. Therefore, if $P < 0.05$, the two sets of data have
327 different means, otherwise the two means have no significant statistical difference.

328

329

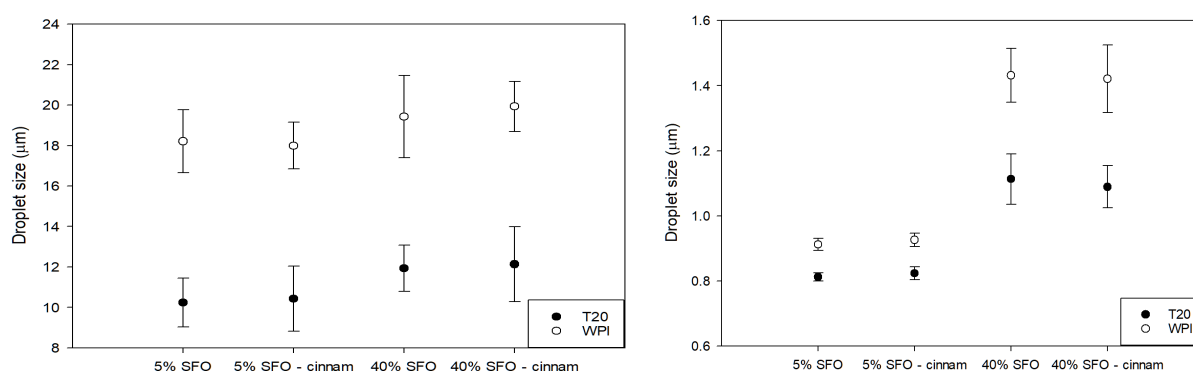
330 3. Results and discussion

331 3.1. Droplet size analysis and zeta-potential measurements

332 The o/w emulsions containing 5% or 40% w/w SFO (dispersed phase) and stabilised by either
333 T20 or WPI, were studied both in the presence and absence of included cinnamaldehyde;
334 comprising 0.7% of the total oil fraction in each system. Emulsifier concentration was fixed at
335 1% w/w, which was sufficient to stabilise the emulsions. It was important to characterise the
336 simple emulsions before conversion to emulsion gels, for printing, in order to establish a
337 baseline for later stability testing (see section 3.3). These systems were all formed in
338 dimensions that were able to undergo printing through the 0.8 mm nozzle, practically
339 undisturbed. They were also determined to be stable during the heating step described in
340 section 2.3, and thus are not expected to change from production to printing. We were able to
341 deliver systems with controlled variations to droplet length scale, emulsifier type, dispersed
342 phase content as well as droplet surface charge. Establishment of this level of customisation
343 is important for proving the use of 3DP κ C-emulsion gels as flexible delivery systems for
344 targeted molecules. $D_{4,3}$ droplet size data is presented in Fig. 2A for larger scale and Fig 2B
345 for smaller scale emulsions.

346 Statistical analysis showed no significant difference in the droplet sizes produced by varying
347 the SFO concentration or by replacing 0.7% of the oil fraction with cinnamaldehyde, in either
348 length scale. However, there is a statistically significant difference between the emulsions
349 stabilised by T20 compared to those stabilised by WPI. This is due to T20 being a low
350 molecular weight surfactant (LMWS), meaning it can position itself faster at the interface
351 (Kenta, et al., 2013). Furthermore, LMWS are superior at decreasing the water/oil interfacial
352 tension compared to proteins, further aiding droplet breakup (Beverung, Radke, & Blanch,
353 1999).

354



355

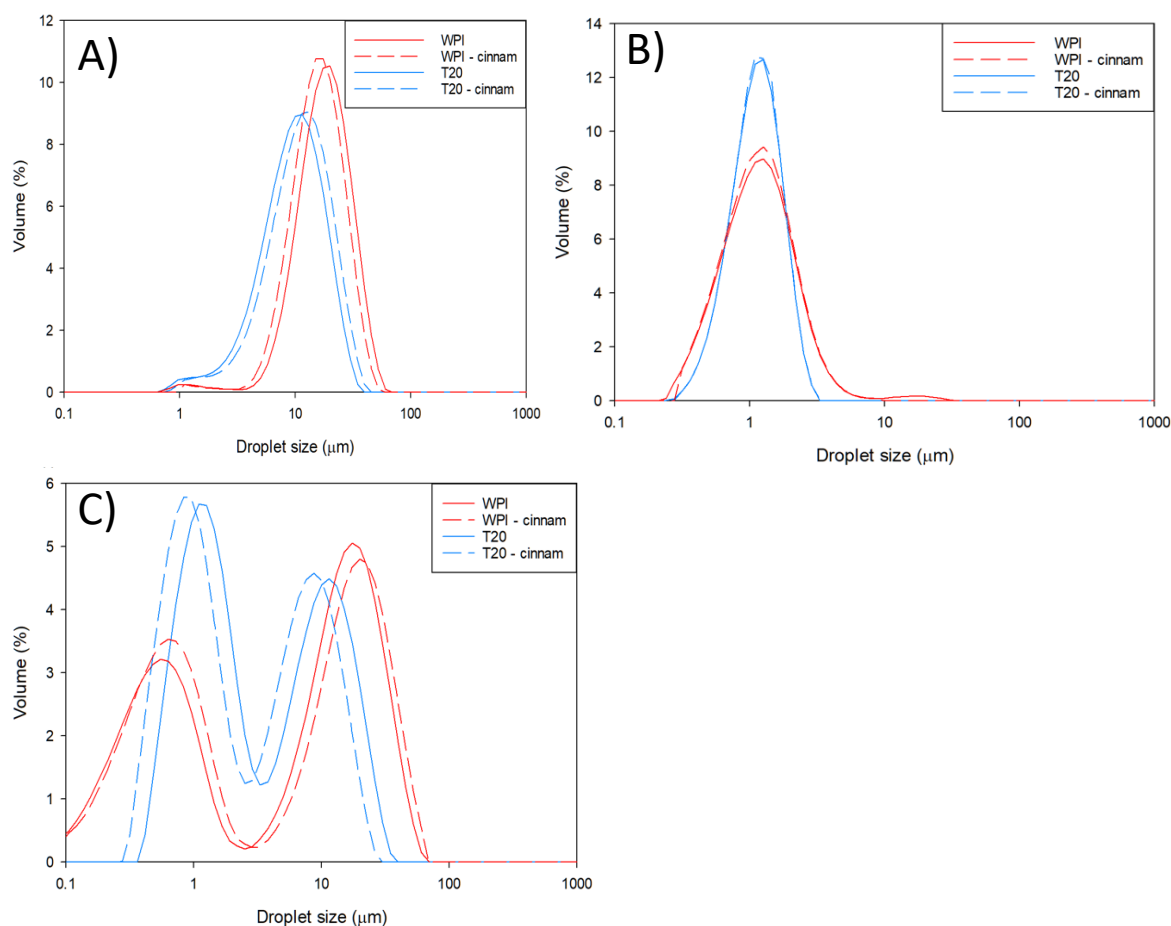
356 *Figure 2: Comparison of the average droplet size of emulsions with and without cinnamaldehyde containing 5%*
357 *or 40% SFO and 1% w/w T20 or WPI in the larger (A) or smaller (B) scale.*

358

359 Bimodal distributions were also created to be used for stability testing (see section 3.3), with
360 larger and smaller scale emulsions mixed in a 1:1 ratio. Because release studies were to be
361 carried out, the possibility of custom release profiles created by mixing different droplet sizes
362 in varying ratios, meant that purpose made bimodal droplet distributions were investigated as
363 well. However, since the $D_{4,3}$ values from the mastersizer are calculated for monomodal
364 distributions, it is far more practical to show the droplet distribution graphs, which are given in
365 Fig 3, including the distributions of the individual size scale systems. This is because a single,
366 average droplet size value does not fully represent the equally mixed droplet populations for
367 the mixed size scale systems.

368 The distributions in Fig. 3 showed that there was no significant difference when replacing 0.7%
369 of the oil fraction with cinnamaldehyde. Furthermore, the mixing of the larger and smaller scale
370 emulsions created bimodal distributions, which reflected the two constituent monomodal
371 distributions of which they were comprised.

372



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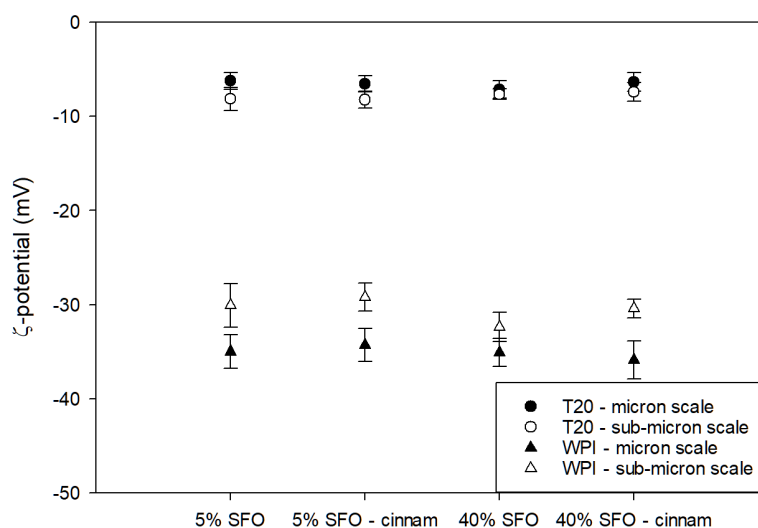
375 *Figure 3: $D_{4,3}$ distribution of 40% SFO (A) larger scale emulsions, (B) smaller scale emulsions and (C) mixed scale*
376 *emulsion systems stabilised with either T20 or WPI.*

377

378 The emulsions were also tested for their ζ -potential, to assess whether the different
379 emulsifiers, the changes in length-scale of the emulsion droplets, or the addition of the
380 cinnamaldehyde fraction has an effect on surface charge (Fig 4). The ζ -potential values for
381 T20 reflect that it is a non-ionic surfactant, which stabilises emulsions through steric repulsion,
382 rather than surface charge (Teo, et al., 2016). The WPI stabilised emulsions had a strong

383 negative charge, owing to being above their isoelectric point, giving a ζ -potential value of
 384 around -34 mV to -37mV for larger-scale emulsions and a statistically significant difference of
 385 -29mV to -32 mV for the smaller-scale emulsions. This is fairly typical with smaller droplet
 386 sizes having been shown to present with a lower ζ -potential value (A. Wiącek & Chibowski,
 387 1999; A. E. Wiącek & Chibowski, 2002). Replacing 0.7% of the SFO fraction with
 388 cinnamaldehyde did not have a statistically significant difference on the ζ -potential of the
 389 emulsions.

390



391

392 *Figure 4: ζ -potential of O/W emulsions with and without cinnamaldehyde, stabilised by either T20 or WPI in the*
 393 *micron and sub-micron scale*

394

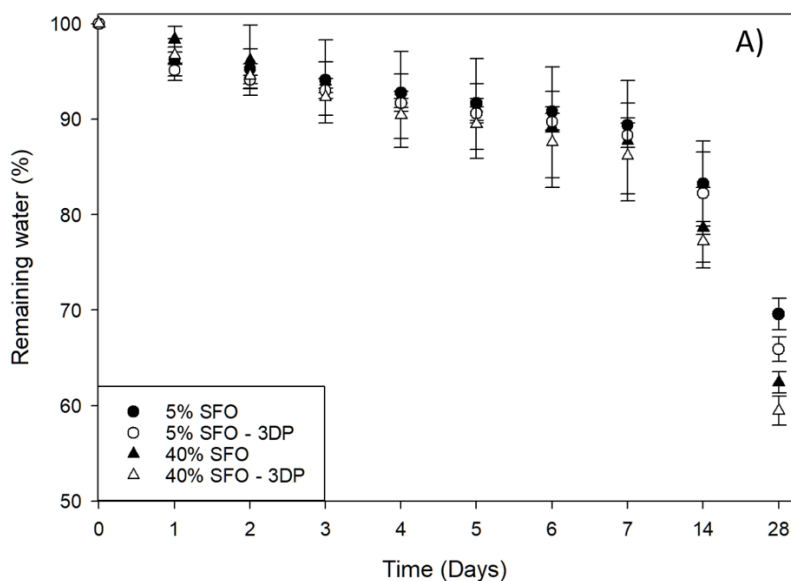
395 3.2.Syneresis testing

396 During emulsion gel formation, while most water is retained within the gel network overtime,
 397 some is expelled during contraction over time as the polymer helices aggregate further
 398 (Thrimawithana, Young, Dunstan, & Alany, 2010). Syneresis is considered to be unfavourable
 399 when it comes to the use of biopolymer gels as molecular delivery vehicles, as any loss of
 400 water could lower the availability of any hydrophilic molecules within the water phase of the
 401 gels. Fig. 5 shows the results of syneresis testing over 28 days for cast and 3DP κ C-emulsion
 402 gel cuboids containing 5% or 40% SFO stabilised by either T20 or WPI. The values have been
 403 normalised for the total water content.

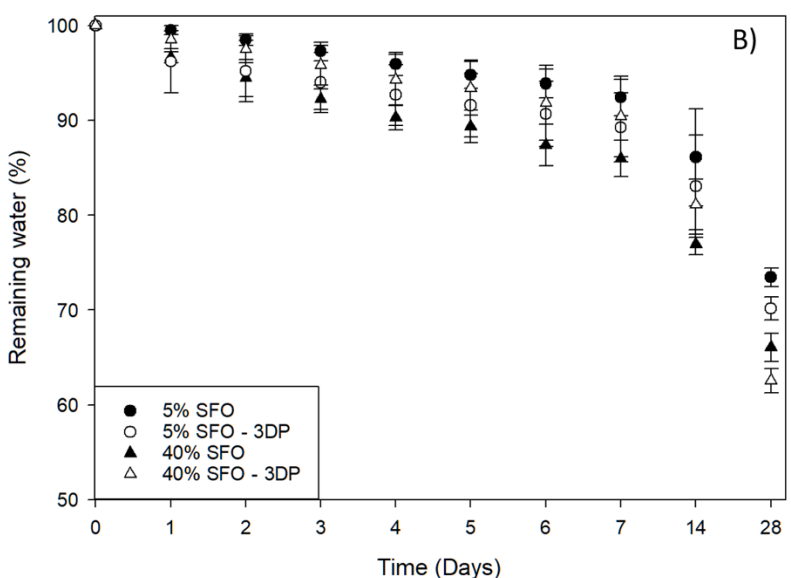
404 As the gels continued to contract over time, water is expelled from within the gel matrix. The
 405 syneresis of the emulsion gels followed the same pattern, regardless of whether they were
 406 stabilised by T20 or WPI. Previous studies have shown that an increase in SFO concentration
 407 within emulsion gels reduces the water loss from syneresis; however, this study did not fix the
 408 biopolymer concentration to the water content (Hongqiang Chen, Lu, Yuan, Gao, & Mao,
 409 2021). Therefore as the oil fraction increased, there was an effective increase of the
 410 biopolymer concentration within the aqueous phase of the emulsion gels. Furthermore while
 411 the data presented in Fig. 5 went to 40% w/w SFO, the previous study only assessed emulsion
 412 gels up to 20% SFO, making comparisons more difficult with previous reported results in
 413 literature. Here, there was no statistically significant difference between 5% and 40% SFO κ C-
 414 emulsion in terms of water loss before 28 days had passed, then a statically significant
 415 difference was observed. One explanation for this could be the far higher oil concentration
 416 within the 40% SFO emulsion gels, causing greater disruption the network structure, leading

417 to a decrease in gel elasticity (D. Julian McClements, Monahan, & Kinsella, 1993). A decrease
 418 in elasticity of κ C-emulsion gels has been shown to increase the rate of syneresis (Rostami,
 419 Nikoo, Rajabzadeh, Niknia, & Salehi, 2018). The authors have previously reported that 40%
 420 SFO κ C-emulsion gels have lower elasticity compared to 5% SFO κ C-emulsion gels (Kamlow,
 421 Spyropoulos, et al., 2021). The 3DP κ C-emulsion gels lost more water than their cast
 422 equivalents, and this was believed to be due to the layering that runs through the 3DP κ C-
 423 emulsion gels, as a consequence of the 3DP process, which can allow water a shorter route
 424 to exit the gels (Kamlow, Vadodaria, et al., 2021).

425



426



427

428 *Figure 5: Syneresis of the κ C-emulsion gels with different oil fractions for 3DP and cast κ C-emulsion gels stabilised*
 429 *by (A) T20 and (B) WPI*

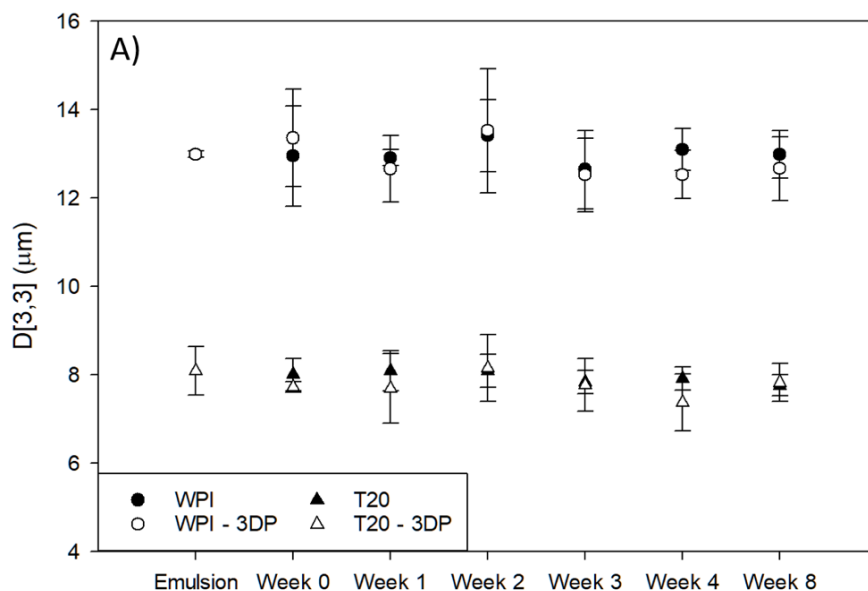
430

431 3.3. Time Domain-NMR stability testing

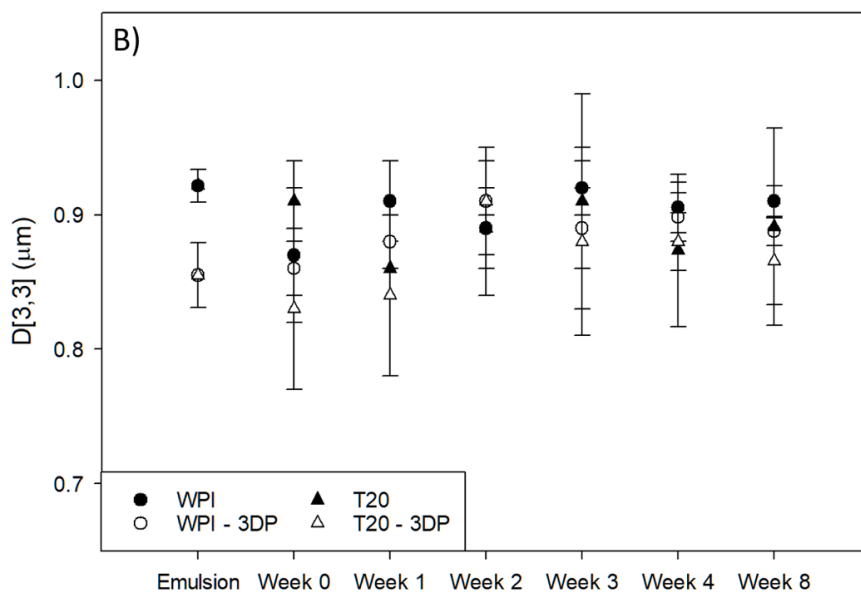
432 Time Domain-NMR (TD-NMR) was used to evaluate the droplet size within κ C-emulsion gel
 433 samples containing monomodal and bimodal droplet distribution systems in both the micron

434 and sub-micron scale. This was to assess whether the 3DP process, causes aggregation or
 435 flocculation of the emulsion systems, once gelled. TD-NMR has the advantage of being able
 436 to evaluate droplet size distributions within solid systems, unlike dynamic light scattering
 437 techniques and without the time-consuming nature of microscopy. Fig 6. Shows the data for
 438 emulsion gels over 8 weeks for micron and sub-micron, monomodally distributed κ C-emulsion
 439 gel samples.

440



441



442

443 *Figure 6: TD-NMR over 8 weeks showing $D_{3,3}$ values for cast and 3DP κ C-emulsion gels in the micron (A) and sub-*
 444 *micron (B) scales*

445

446 Fig. 6 showed that despite the simple emulsions being heated and stirred for two and a half
 447 hours during emulsion gel production, there was no significant coalescence of the oil droplets
 448 observed. Furthermore, the 3D printing process also appeared to have no significant effect on
 449 the oil droplet size within the κ C-emulsion gels. The differences observed between the T20

450 and the WPI follow trends observed from section 3.1. While the droplet sizes appear to have
 451 stayed constant for both emulsifiers, the nature of TD-NMR means that it does not detect
 452 flocculation of oil droplets, as the restriction of self-diffusion of oil by the walls of the droplets,
 453 won't be affected by the flocculation (Goudappel, van Duynhoven, & Mooren, 2001). However,
 454 previous studies have shown that κ C-emulsion gels flocculate when stabilised by T20
 455 (Kamlow, Spyropoulos, et al., 2021; H. Singh, Tamehana, Hemar, & Munro, 2003). This
 456 doesn't occur with the WPI stabilised κ C-emulsion gels, and is linked to the higher surface
 457 charge on the oil droplets stabilised by WPI (see Fig. 4). This highlights the need to examine
 458 complex systems such as emulsion gels through various techniques. There was no change
 459 seen over 8 weeks, which is due to the solid continuous phase restricting any movement of
 460 the oil droplets within the network.

461 Separately sub-micron and micron scale emulsions were created and mixed by stirring for five
 462 minutes and then gelled as above. These were also stored for 8 weeks and the droplet sizes
 463 examined. Fig. S1 and table 1 show that despite intentionally creating a bimodal emulsion
 464 distribution, the emulsion gels maintained their stability over 8 weeks. This is despite the fact
 465 that bimodal emulsions are less stable, and more prone to Ostwald ripening and coalescence
 466 (van der Ven, Gruppen, de Bont, & Voragen, 2001). The addition of cinnamaldehyde caused
 467 no significant change to the droplet sizes. Since $D_{3,3}$ are calculated based on monomodal
 468 distributions, these values alone are not fully representative of the droplet size distributions
 469 present within these systems (Juslin, Antikainen, Merkku, & Yliruusi, 1995). It also appears
 470 that the tween emulsion gel systems appear to have eliminated the flocculation observed in
 471 the simple emulsion systems. The distribution values over 8 weeks are presented in table 1.

472 Table 1 shows that the emulsion gels for the mixed-scale systems maintained the same
 473 distributions and σ values after production and storage for 8 weeks. The σ value corresponds
 474 to the standard deviation of the logarithm of the droplet diameter. The κ C-emulsion gels
 475 stabilised with T20 had a narrower droplet size distribution owing compared to those stabilised
 476 by WPI, which is reflected in the smaller σ value. The demonstrated ability of emulsion gels to
 477 maintain emulsion stability over time, coupled with their thermoreversible nature means that
 478 they could potentially be used to store poorly stable emulsions as emulsion gels, and then
 479 heat them to undergo a gel-sol transition and consume them as a liquid form if needed.

480

481 *Table 1: Data on droplet size distributions of mixed scale κ C-emulsion gels following production and after 8*
 482 *weeks. A higher σ value indicates a wider droplet size distribution*

	Droplet size after 0 weeks				Droplet size after 8 weeks			
	WPI	T20	WPI with cinnamaldehyde	T20 with cinnamaldehyde	WPI	T20	WPI with cinnamaldehyde	T20 with cinnamaldehyde
Diameter 2.5% (μm)	0.31 \pm 0.05	0.25 \pm 0.04	0.30 \pm 0.04	0.23 \pm 0.02	0.28 \pm 0.07	0.25 \pm 0.02	0.24 \pm 0.05	0.24 \pm 0.01
Diameter 50% (μm)	5.80 \pm 0.23	2.89 \pm 0.15	5.79 \pm 0.30	3.03 \pm 0.09	5.83 \pm 0.33	2.96 \pm 0.02	5.72 \pm 0.40	3.00 \pm 0.04
Diameter 97.5% (μm)	81.88 \pm 4.21	47.27 \pm 2.23	77.61 \pm 2.14	47.66 \pm 3.19	79.37 \pm 3.86	48.46 \pm 1.69	77.45 \pm 3.61	46.34 \pm 1.82
σ	2.54 \pm 0.06	1.53 \pm 0.08	2.55 \pm 0.10	1.64 \pm 0.12	2.61 \pm 0.09	1.42 \pm 0.36	2.59 \pm 0.10	1.51 \pm 0.09

483

484 3.4. Texture profile analysis

485 Texture profile analysis of gels is an important technique for characterising the gel
486 microstructure. In the past TPA has been used to assess how gels perform when it comes to
487 the release of molecules from within their matrices. More rigid, and less elastic gels tends to
488 release molecules slower, owing to a more dense gel network retarding molecular release
489 (Boland, Delahunty, & van Ruth, 2006; Özcan, et al., 2009). While previous studies have
490 carried out TPA on 3DP gels, there exists little, if any, literature that utilises penetration testing,
491 with all the existing work focusing on compression testing (Kamlow, Spyropoulos, et al., 2021;
492 Kamlow, Vadodaria, et al., 2021; Strother, Moss, & McSweeney, 2020; Fanli Yang, Zhang,
493 Bhandari, & Liu, 2018).

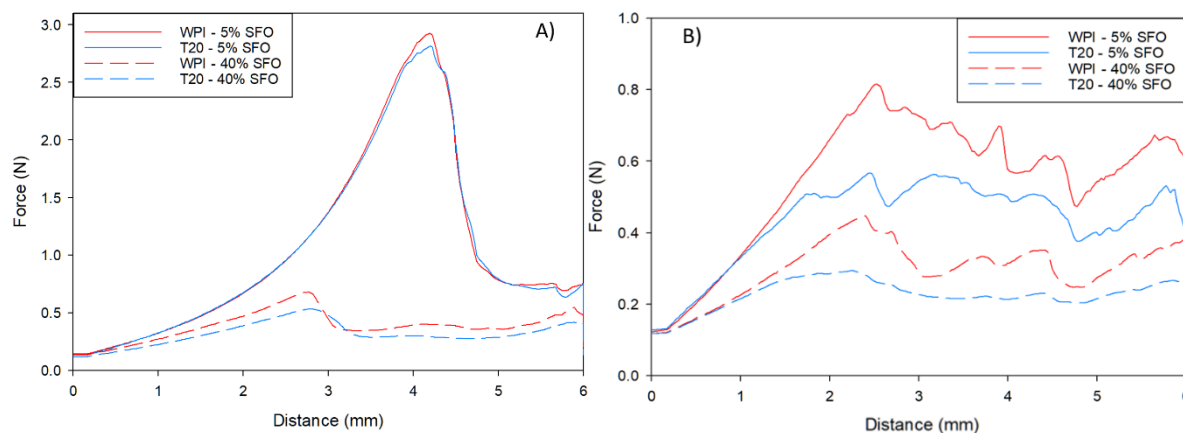
494 Since penetration testing measures force as a function of penetration depth, it is much more
495 sensitive to local variances in the gel architecture. Whereas, compression tests are
496 determined by the average material property for the whole sample (C. M. Lee & Chung, 1989).
497 Penetration testing data for both the 3DP and cast κ C-emulsion gels studied here are shown
498 in Fig. 7. Cast κ C-emulsion gels display a typical shaped TPA curve, with one failure point
499 (Fig. 7A), whereas 3DP gels had several peaks and troughs, with each of these roughly
500 corresponding to a penetration depth of 1.2 mm (Fig. 7B); this is also compatible with the
501 printed layer height. The data in Fig. 7 also shows (for both cast and 3DP κ C-emulsion gels)
502 that as the concentration of SFO increases, the amount of force required to penetrate the gels
503 decreased. Literature suggests this behaviour to be a result of the oil droplets within the
504 network acting as non-interacting filler particles, with therefore further increases to their
505 population (higher SFO content) resulting in a more pronounced disruption in the formation of
506 the gel network around them, and thus weaker gels (D. Julian McClements, et al., 1993).

507 A previous study by the present authors (Kamlow, Spyropoulos, et al., 2021) revealed no
508 statistically significant difference in the performance of 3DP κ C-emulsion gels when
509 undergoing compression tests, regardless of SFO concentration. It was observed that under
510 compression, 3DP gels undergo delamination, breaking down at the semi-fused sites that
511 follow the lines of the printing, as opposed to cast gels which are one continuous network.
512 However, the data for the penetration testing in Fig. 7 highlights that penetration testing can
513 demonstrate a difference in performance for 3DP gels; not only was a difference observed
514 with SFO concentration, but also as a function of the emulsifier chosen. The disparity in the
515 results between compression and penetration testing is due to how failure propagates when
516 gels are subjected to either type of forces. While compression testing evaluates the
517 cohesiveness of the gels, that is to say their overall binding, penetration testing assesses the
518 degree of compactness in the gels, that is to say, their density (C. M. Lee, et al., 1989).

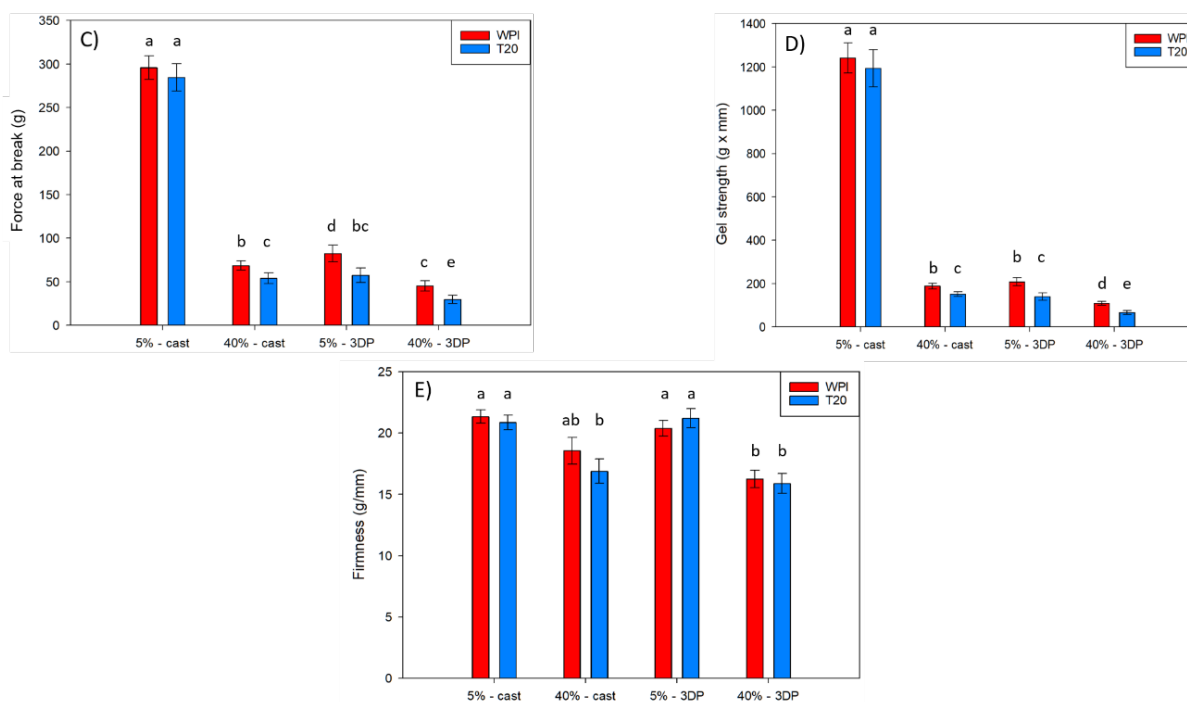
519 From the force-distance graphs in Fig. 7A and 7B, data for force at break, in g, firmness, in
520 g/mm, and gel strength, in g x mm, could be determined and these are shown in Fig. 7C-E.
521 The 5% SFO cast gels had far higher values for force at break and gel strength because they
522 lacked the extensive network disruption caused by either a high concentration of SFO or the
523 discontinuous nature of a 3DP bulk structure. This was responsible for the far higher values
524 observed in Fig. 7C and 7D for 5% SFO cast gels. In terms of force at break and gel strength
525 (Fig. 7C and D), there was a statistically significant difference between the emulsion gels
526 stabilised by either T20 or WPI, except for the cast 5% SFO gels. WPI contains 3.16%, milk-
527 based ash contains cations such as potassium and calcium which are known to reinforce κ C
528 gels (Hermansson, et al., 1991). It is believed that this was a contributing factor, to the WPI-
529 stabilised emulsion gels requiring more force before failure. The firmness data (Fig. 7E) saw
530 the 5% SFO gels having no significant difference between them, regardless of emulsifier. For
531 the 40% SFO cast gels, there was no significant difference between the cast gels stabilised
532 by WPI, and the 5% SFO gels. While the 40% SFO cast gels stabilised by T20 were statistically
533 significantly different from the 5% SFO gels, but not the 40% SFO cast gel stabilised by WPI.
534 Between the 40% SFO 3DP gels there was no statistically significant difference, nor was there
535 one with the 40% SFO cast emulsion gel stabilised by T20, but there was for the 40% SFO

536 cast gel stabilised by WPI. The lower firmness values of the 40% SFO gels, both cast and
 537 3DP, suggest that these systems deformed more easily and tended to flow more before
 538 breaking, when compared to the 5% SFO gels (Pang, Deeth, Sopade, Sharma, & Bansal,
 539 2014).

540



541



542

543 *Figure 7: Force-time graphs for (A) cast and (B) 3DP κC-emulsion gels stabilised by T20 and WPI. (C) Force at*
 544 *break, (D) Gel strength and (E) Firmness of the 3DP and cast κC emulsion gel cuboids containing emulsions*
 545 *stabilised by T20 and WPI. Letters represent statistical significance (P<0.05)*

546

547 3.5. Release studies

548 The amount of cinnamaldehyde released from 3DP and cast κC-emulsion gels was measured
 549 at 37 °C and 20 °C in water as a simple release medium and PBS and 0.1M HCl as they are
 550 more physiologically relevant release media. Control cinnamaldehyde release performance
 551 from non-gelled emulsions and a pure sunflower oil phase (both) in water at 37 °C, was also
 552 assessed. The release data acquired are all presented in Fig 8 and Fig S2.

553 The release data showed no major difference in the amount of cinnamaldehyde being released
554 from emulsion gels, regardless of whether they were 3DP or cast. This differed from our
555 previous study which found that 3DP gels released a greater proportion of their active in the
556 same timeframe compared to cast gels (Kamlow, Vadodaria, et al., 2021). However, the
557 previous study was for a hydrophilic active directly encased within the gel network, while the
558 present work studies the release of cinnamaldehyde (which is lipophilic) from emulsion gels.
559 This appears to suggest that in the current study, overall cinnamaldehyde release from both
560 the 3DP and cast κ C-emulsion gels is predominantly dictated by the active's liberation from
561 the oil droplets rather than by its subsequent discharge from the surrounding gel network. One
562 difference between 3DP and cast gels is observed in Fig. 8A to 8F. Here, there is a divergence
563 in the release performance between the 3DP and cast κ C-emulsion gels that is exhibited
564 around the 60-120 mins time frame. Placing the gels into the acceptor medium, creates an
565 osmotic gradient for the transfer of cations from the gel into the aqueous sink; this is controlled
566 by diffusion. This took place at a faster rate in the 3DP (than in the cast) gels, as their inherent
567 layered structure facilitates the migration of the cations into the acceptor phase (Kamlow,
568 Vadodaria, et al., 2021). However, when eventually the gel network of the cast assemblies is
569 also lost, cinnamaldehyde release from these systems is seen to once again coincide with that
570 from their 3DP counterparts. This divergence takes place at later stages in the release
571 experiments utilising PBS as the acceptor phase, owing to the greater concentration of ions
572 (compared to deionised water) present in this case.

573 In order to confirm that the loss of gelling cations via diffusion causes the collapse of the gels,
574 the data for cinnamaldehyde release in 1M KCl was scrutinised (Fig. S2D). Furthermore
575 cinnamaldehyde release at 20 °C (Fig. S2A-C) was carried out to assess the effect of
576 temperature. In both cases, the previously observed divergence between 3DP and cast κ C-
577 emulsion gels was absent, either because the concentration gradient led to the gels taking up
578 salt (1M KCl) or because the lower temperature (20 °C) has slowed down the diffusional
579 transfer of salts out of the gel network (Vrentas & Vrentas, 1992). This led to release rates
580 being lower in PBS as seen in Fig. 8C-D compared to 19A-B. In 0.1M HCl (Fig. 8E and 8F), a
581 statistically significant increase in the percentage release of cinnamaldehyde was observed.
582 This was due to the free carbonyl group present on cinnamaldehyde, facilitating the formation
583 of Schiff base adducts with an increased aqueous solubility (Friedman, 2017; Wei, Xiong,
584 Jiang, Zhang, & Wen, 2011). In terms of cinnamaldehyde release in an acidic environment
585 (0.1M HCl), WPI stabilised emulsion gel systems behaved differently to those stabilised by
586 T20. As the pH in this case is below the isoelectric point of WPI, the protein has an overall
587 positive charge (Chanamai & McClements, 2002), and thus can associate with κ C, effectively
588 acting as a gelling cation (de Kruif, Weinbreck, & de Vries, 2004; Stone & Nickerson, 2012).
589 This meant that the cast and 3DP gels remained solid, despite any potential loss of gelling
590 cations such as potassium and sodium to the acceptor phase. This yielded the different
591 release behaviour compared to the remaining release studies at 37 °C. The enhanced
592 aqueous solubility of cinnamaldehyde within an acidic environment can be seen through
593 comparison of Fig. 8E and 8F with Fig. 8A-D, with the release in 0.1M HCl yielding a
594 statistically significant increased amount of cinnamaldehyde compared to release in other
595 media; this trend persists at 20 °C as well as shown in the supplementary information. This
596 shows that simply by controlling the emulsifier used to stabilise the κ C-emulsion gels, the
597 release rate of active molecules can be manipulated based on the release medium. The
598 enhanced solubility and release of cinnamaldehyde in 0.1M HCl compared to water, was also
599 observed when the active was simply delivered by dissolution in a pure SFO phase, as seen
600 in Fig. S2F. Here there was again, a statistically significant difference in release based on the
601 release medium tested (0.1M HCl or water).

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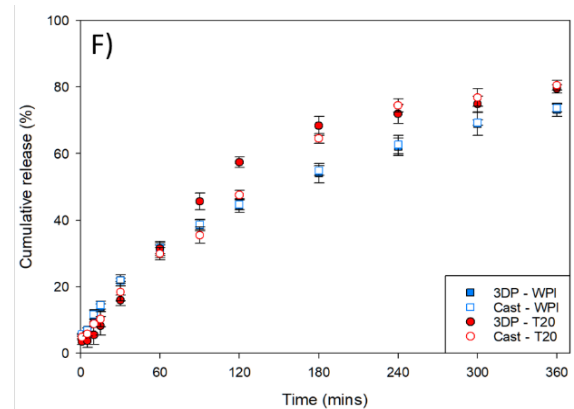
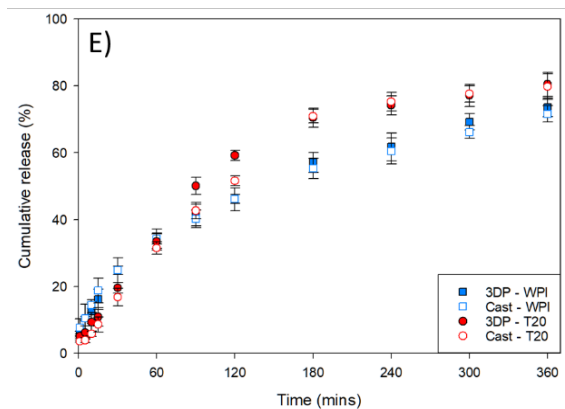
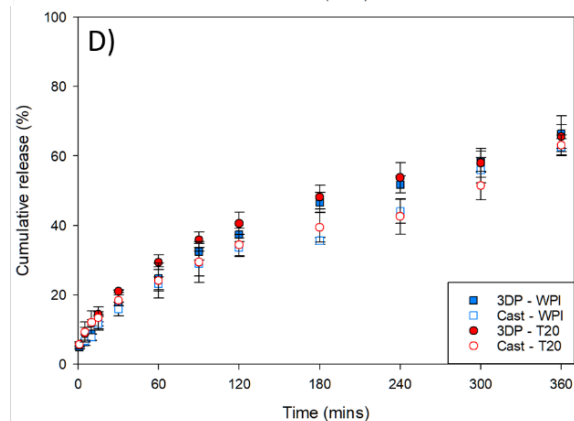
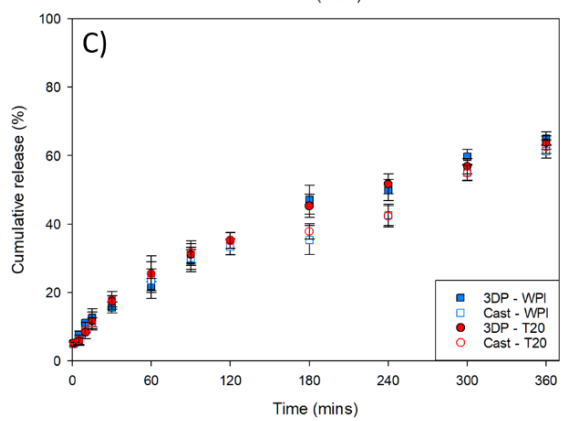
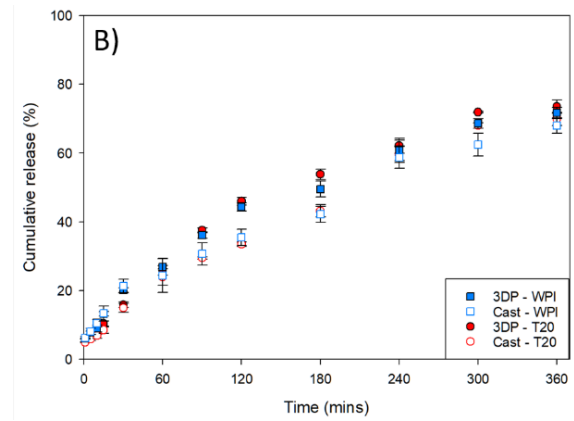
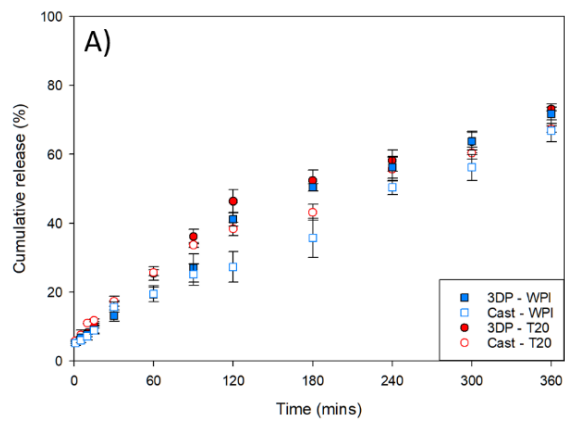


Figure 8: A comparison of cumulative release rates of cinnamaldehyde from 3DP and cast κ C-emulsion gels at 37 °C in water stabilised by T20 and WPI in the (A) micron and (B) sub-micron scale and the same order is followed for the remaining figures with (C-D) in PBS, (E-F) in 0.1M HCl

Another observation was that there was no significant difference in the trends for final release concentrations observed between the micron and sub-micron scale emulsions gels, and this held true even for the simple emulsion systems in Fig. S2E. Even though a smaller average droplet size yields an increased surface (interfacial) area, that should accelerate release out of the oil globules, literature in this area includes a number of conflicting results (Li & McClements, 2010). Some studies have reported that such a droplet size reduction yields an increase in percentage release of lipophilic molecules (Charles, Lambert, Brondeur, Courthaudon, & Guichard, 2000; S. J. Lee & McClements, 2010), whereas others suggest that droplet size variations have no significant difference (Ahmed, Li, McClements, & Xiao, 2012). Additionally, the use of dialysis tubing has been shown to act as a rate limiting step for the release of lipophiles from emulsion systems, and this may have contributed in not observing

621 a statistically significant difference between the micron and sub-micron scale systems
 622 (Magalhaes, Cave, Seiller, & Benita, 1991). Finally, the possibility exists that κ C and
 623 cinnamaldehyde interactions via hydrogen bonding could have impeded the release of the
 624 active from the gels (Yamada & Shizuma, 2021). The final cinnamaldehyde release values
 625 for the release data are presented in Table 2.

626

627 Table 2: Final cinnamaldehyde release values as a %. Superscript letters indicate statistical significance ($P < 0.05$)

Cinnamaldehyde-carrying system	Acceptor Phase	Temp (°C)	Cinnamaldehyde cumulative release (%) after 6 hours							
			Micron				Sub-micron			
			T20		WPI		T20		WPI	
pure SFO phase	Water	37	82.9 ^a							
	0.1M HCl	37	89.3 ^b							
O/W emulsion	Water	37	76.8 ^c		76.3 ^c		77.7 ^c		77.8 ^c	
			3DP	Cast	3DP	Cast	3DP	Cast	3DP	Cast
O/W emulsion gel	Water	37	71.2 ^d	68.4 ^d	71.4 ^d	68.8 ^d	71.5 _d	69.9 ^d	71.7 ^d	69.0 ^d
		20	55.6 ^e	58.3 ^e	56.2 ^e	60.1 ^e	-	-	-	-
	PBS	37	63.7 ^f	62.6 ^f	65.0 ^f	61.2 ^f	64.6 ^f	63.1 ^f	64.4 ^f	62.6 ^f
		20	53.1 ^g	53.5 ^g	51.9 ^g	52.2 ^g	-	-	-	-
	0.1M HCl	37	80.5 ^h	79.8 ^h	74.6 ⁱ	74.6 ⁱ	79.5 _h	80.5 ^h	74.2 ⁱ	74.7 ⁱ
		20	66.4 ^f	69.4 ^{df}	66.0 ^f	66.2 ^f	-	-	-	-
1M KCl	37	65.1 ^f	67.5 ^f	63.6 ^f	64.5 ^f	-	-	-	-	
O/W emulsion gel*	Water	37	69.3 ^d	69.1 ^d	68.8 ^d	68.7 ^d	-	-	-	-

628 *This system was used for the co-release of cinnamaldehyde (cinn; enclosed within the oil droplets of
 629 the O/W emulsion gels) and erioglaucine disodium salt (EDS; entrapped within the gel network of the
 630 O/W emulsion gels)

631

632 The co-release profiles for cinnamaldehyde and EDS from both the printed and cast κ C-
 633 emulsion gels and for all release media, were fitted to Ritger-Peppas model shown in Eq [3]
 634 in order to assess the contributions of diffusion and relaxation to the release of the active
 635 molecules (Ritger, et al., 1987). The exponent m values resulting from the fits are given in
 636 Table 3. The data suggests cinnamaldehyde release from 3DP cylinders is driven primarily by
 637 Fickian diffusion as opposed to relaxation of the polymer chains. This is believed to be due to
 638 the differences in the internal bulk structures of the 3DP cylinders allowing water to penetrate
 639 faster into the 3DP shapes compared to cast gel structures. This leads to a greater diffusion
 640 contribution to release compared to the relaxational contribution (Falk, Garramone, &
 641 Shivkumar, 2004; Kamlow, Vadodaria, et al., 2021). This is demonstrated by the cast gels
 642 having m values further away from 0.5, indicating that the relaxational contribution is amplified.
 643 This supports the notion that water was unable to penetrate into the cast cylinders as quickly
 644 as the 3DP cylinders, meaning that the relaxation of the polymer chains played a bigger part
 645 in the release of the actives.

646 This modelling data further supports some of the previous conclusions made about differences
 647 in release of cinnamaldehyde from micron and sub-micron scale oil droplets. The different
 648 droplet length scales did not see a significant difference in cinnamaldehyde release with
 649 regards to diffusion and relaxational contributions. The release tests carried in 0.1M HCl,
 650 showed that the WPI had a larger relaxational contribution, most likely due to the fact that the
 651 network remained intact owing to the WPI molecules stabilising the κ C-gel network. The
 652 release tests carried out at 20 °C had larger m values owing to the decreased energy in the
 653 system, slowing the diffusion contribution, and this meant that there was as delay as relaxation

654 had to come into effect to drive more of the release. In terms of the co-release experiments,
 655 the presence of the EDS releasing had no effect on the observed release phenomena of the
 656 cinnamaldehyde. This provides further evidence that their release occurred through two
 657 different mechanisms. The *m* values for EDS release were higher for the cast gels, compared
 658 to the 3DP gels. While our previous study highlighted a much starker difference between 3DP
 659 and cast gels for release of a hydrophilic molecule, there were differences such as the lack of
 660 dialysis tubing and the previous active, vitamin B1 being cationic, meaning it complexed with
 661 the κ C which would have affected its release rate (Kamlow, Vadodaria, et al., 2021). 1M KCl
 662 did not have a significant effect on the *m* value compared to the other release media, showing
 663 that the gels turning to liquid did not necessarily affect the contributions of diffusion and
 664 relaxation to the release. This is highlighted by the similar values observed with the simple
 665 emulsions. This further supports the release of the cinnamaldehyde primarily occurring
 666 because of expulsion from the oil droplets, rather than the gel network itself.

667

668 *Table 3: Data on the exponent *m*, which indicates the balance between the relaxational and diffusional*
 669 *contribution to the release of cinnamaldehyde and EDS.*

Active-carrying system	Acceptor Phase	Temp (°C)	Exponent <i>m</i> ± SD (<i>R</i> ²)							
			Micron				Submicron			
			T20		WPI		T20		WPI	
<i>O/W emulsion</i>	Water	37	0.55±0.04 (0.99)		0.57±0.04 (0.99)		0.52±0.05 (0.97)		0.51±0.04 (0.98)	
			3DP	Cast	3DP	Cast	3DP	Cast	3DP	Cast
<i>O/W emulsion gel</i>	Water	37	0.58±0.03 (0.98)	0.66±0.03 (0.99)	0.58±0.06 (0.98)	0.69±0.03 (0.98)	0.55±0.04 (0.99)	0.65±0.04 (0.99)	0.53±0.03 (0.99)	0.67±0.04 (0.97)
		20	0.65±0.02 (0.99)	0.78±0.02 (0.99)	0.66±0.02 (0.99)	0.72±0.02 (0.99)	-	-	-	-
	PBS	37	0.53±0.02 (0.99)	0.67±0.03 (0.98)	0.55±0.03 (0.99)	0.69±0.03 (0.98)	0.55±0.03 (0.99)	0.67±0.03 (0.98)	0.62±0.01 (0.99)	0.62±0.03 (0.98)
		20	0.72±0.01 (0.99)	0.74±0.02 (0.99)	0.70±0.01 (0.99)	0.73±0.02 (0.99)	-	-	-	-
	0.1M HCl	37	0.58±0.04 (0.99)	0.61±0.04 (0.99)	0.63±0.01 (0.99)	0.66±0.01 (0.99)	0.51±0.01 (0.99)	0.59±0.04 (0.99)	0.63±0.02 (0.99)	0.66±0.02 (0.99)
		20	0.62±0.02 (0.99)	0.65±0.02 (0.99)	0.71±0.01 (0.99)	0.75±0.02 (0.99)	-	-	-	-
	1M KCl	37	0.59±0.02 (0.99)	0.68±0.02 (0.99)	0.58±0.02 (0.99)	0.64±0.02 (0.99)	-	-	-	-
<i>O/W emulsion gel*</i>	Water (cinn.)	37	0.56±0.02 (0.98)	0.69±0.03 (0.98)	0.56±0.03 (0.98)	0.67±0.04 (0.97)	-	-	-	-
	Water (EDS)	37	0.55±0.02 (0.96)	0.62±0.02 (0.98)	0.52±0.03 (0.98)	0.59±0.03 (0.98)	-	-	-	-

670 *This system was used for the co-release of cinnamaldehyde (cinn; enclosed within the oil droplets of
 671 the *O/W* emulsion gels) and erioglaucine disodium salt (EDS; entrapped within the gel network of the
 672 *O/W* emulsion gels)

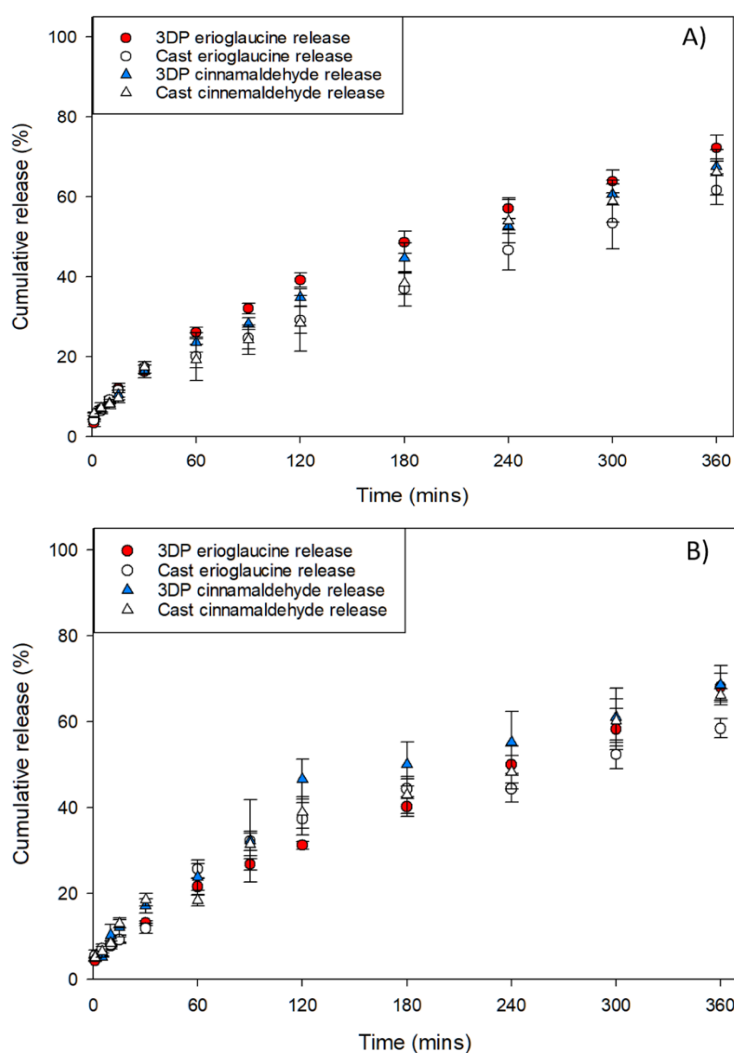
673

674 3.6 Co-release study

675 Co-release κ C-emulsion gels was also evaluated. Two actives were introduced within the κ C-
 676 emulsion gels, with cinnamaldehyde being present within the lipid phase (oil droplets) of the
 677 emulsion gels, and EDS entrapped within the aqueous gel phase. This was carried out to
 678 assess whether co-release of actives placed within separate compartments of the κ C-
 679 emulsion gel microstructure can effectively be utilised to enable their independent co-delivery.
 680 This approach is certainly unique in the 3DP emulsion gel literature, and thus extends the
 681 current capabilities of these systems to also provide opportunities for the design of novel
 682 and/or customisable co-release profiles of both hydrophilic and hydrophobic actives. The

683 release behaviour of the κ C-EDS-cinnamaldehyde emulsion gels in water at 37 °C were
684 studied and the obtained data is shown in Fig. 9.

685



687
688 *Figure 9: Co-release profiles for cinnamaldehyde and EDS from κ C-emulsion gels stabilised by (A) T20 and (B)*
689 *WPI in water at 37 °C.*

690
691
692 The release profiles showed no significant difference between T20 and WPI stabilised κ C-
693 emulsion gels for the co-release of cinnamaldehyde and EDS. Furthermore, there was once
694 again no significant difference in the final release percentages for the cinnamaldehyde, and
695 the divergence and subsequent convergence could be once more be observed. With the EDS
696 however, there was a statistically significant difference between its release between cast and
697 3DP gels after 6 hours. This indicates that the release profiles of the two model actives are
698 independently controlled; cinnamaldehyde release is principally controlled by expulsion from
699 the entrapped oil droplets, while EDS release is dictated by the rate of its liberation from the
700 gel network. This is in agreement with previous studies comparing 3DP to cast κ C gels when
701 releasing a hydrophilic molecule (Kamlow, Vadodaria, et al., 2021). It should be noted,
702 however, that after 24 hours 100% of the EDS had been released from the gel matrices for
703 both cast and 3DP gels. Compared to the 'mono' release of cinnamaldehyde in water at 37
704 °C, the presence of EDS in the co-release formulation did not have any significant effect on
705 the release of the former active from the κ C-emulsion gels. Overall, the data presented here

706 offer clear confirmation that both 3DP and cast κC-emulsion gels can indeed facilitate the
707 independent co-release of one model lipophilic active (cinnamaldehyde) and one model
708 hydrophilic active (EDS). What is further highlighted here is that the desirable individual co-
709 release profiles can be designed separately and then effectively assembled into one 3DP
710 emulsion gel microstructure, without loss of individual release identity/performance, while
711 utilising 3DP's ability to readily alter the size and shape of the desired product, delivering
712 flexible dosing, without the need of additional moulds or tooling that would be required with
713 cast samples.

714
715

716 **4. Conclusions**

717 This study provides further insight into the stability, mechanical and performance
718 characteristics of κC-emulsion gels. 3DP κC-emulsion gels lose slightly more water over time
719 than cast gels, but the 3DP process has no effect on droplet size stability during production or
720 storage of κC-emulsion gels. This study has for the first time highlighted differences between
721 3DP gels based on their SFO concentration and emulsifier type through penetration testing,
722 with lower SFO concentration and the use of WPI as an emulsifier yielding more resistant gels.
723 This study has shown for the first time in release testing, that the release medium has been
724 shown to affect the release rate of lipophilic molecules from cast and 3DP κC- emulsion gels.
725 However, droplet size and production process had no effect on the release rate, although the
726 use of dialysis tubing might have affected this. Finally, co-release of EDS and cinnamaldehyde
727 was carried out, for the first time from a 3DP bulk structure. This shows that 3DP can be used
728 to create customisable κC gels containing hydrophilic and/or lipophilic active molecules, with
729 desired properties tuneable through variances in SFO concentration, shape, emulsifier type
730 and size. Future studies could focus on the addition of a dietarily appropriate protein and
731 carbohydrate concentrations, to give a total food source that can be fortified with active
732 molecules.

733

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737

738 **References**

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SUPPLEMENTARY INFORMATION

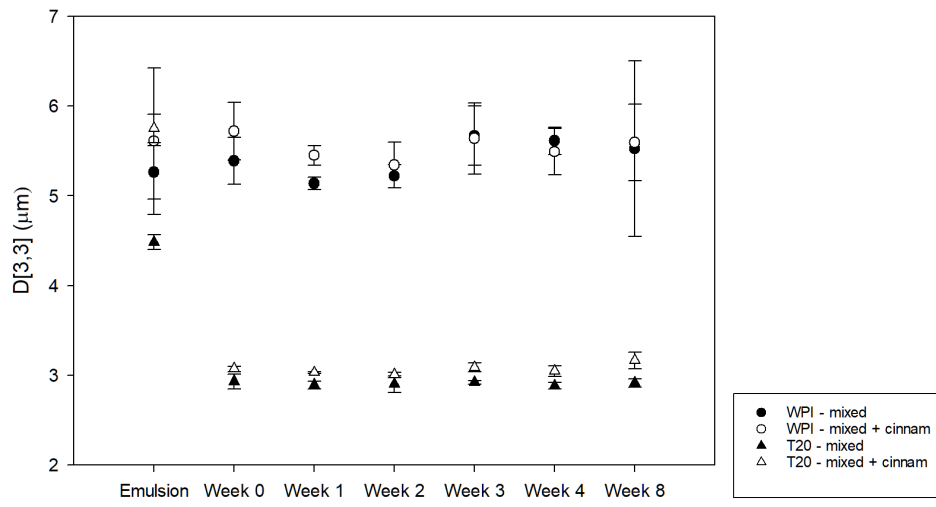


Figure S1: TD-NMR over 8 weeks showing D_{3,3} values for κC-emulsion gels containing mixed micron and sub-micron scale emulsions with and without cinnamaldehyde

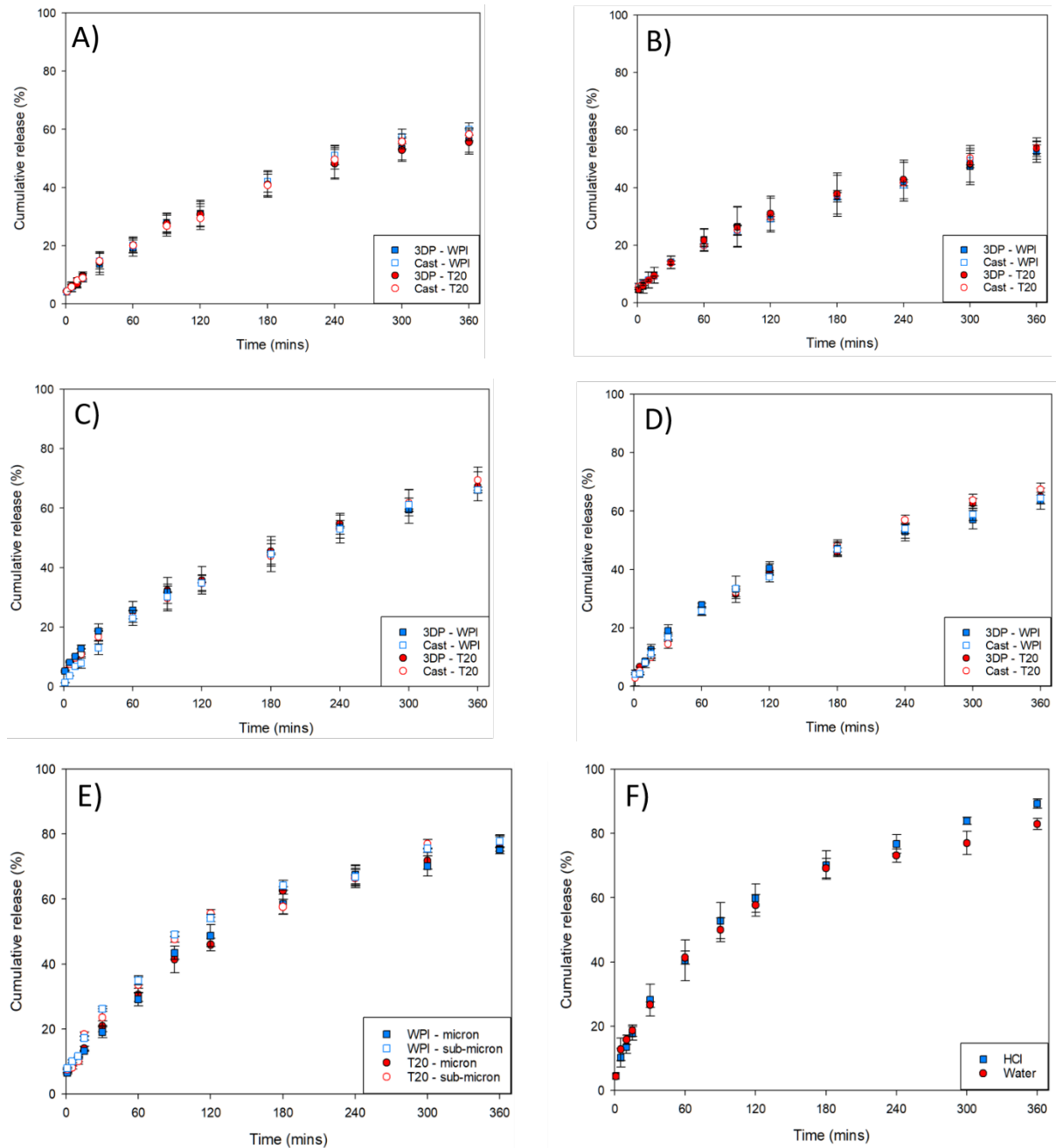


Figure S2: A comparison of cumulative release rates of cinnamaldehyde from 3DP and cast κ C-emulsion gels stabilised by T20 or WPI, at (A) 20 °C in water (B) PBS at 20 °C, (C) 0.1M HCl at 20 °C, (D) 1M KCl at 37 °C, (E) release from non-gelled emulsions in water at 37 °C and (F) release from cinnamaldehyde mixed with SFO in HCl and water at 37 °C